The Mode of Sclerospora sorghi Infection of Sorghum Bicolor Leaves

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ABSTRACT

The mechanism by which *Sclerospora sorghi* penetrates grain sorghum leaves was determined using conidia as inoculum. Germ tubes grew at random on the surface of the leaves and irregular swellings developed in germ tubes at epidermal cell junctions, but no penetration was observed. When a germ tube reached a stoma, elongation ceased and an appressorium formed over the stomatal opening. Once inside, the penetrating structure enlarged to form an oval-shaped substomatal vesicle. This vesicle then gave rise to one or more infection hyphae. Conidia formed during the night, germinated soon after discharge, and penetration was complete by 7:00 AM. Haustoria were observed in host cells 3 hours later. This indicates that *S. sorghi* can enter its host and establish infection in 3 to 4 hours. Phytopathology 61:406-408.

Sclerospora sorghi Weston & Uppal produces oospores in the leaves of grain sorghum (*Sorghum bicolor* [L.] Moench) which survive on crop residue in the soil and serve as primary inoculum for succeeding crops. Conidia, formed on the leaves of primary infected plants, are wind-borne to adjacent plants, penetrate the foliage, and thus account for secondary spread of the pathogen. Although early observations indicated infection by means of conidia (2, 7), this lacked proof for many years as attempts to obtain artificial infection with conidia as inoculum were unsuccessful (10, 11, 12). Safeeulla & Thirumalachar (10) first succeeded in obtaining infection by spraying the foliage with a wetting agent (0.05 sodium ricineolate solution) prior to brushing on the conidia.

Cosper (4) reported appressoria on germ tubes of *S. sorghi* at the junction of epidermal cells of grain sorghum leaves. He also observed subappressorial hyphae within the leaf tissue which indicated direct penetration. The purpose of the present study was to further investigate the mode of penetration, to determine the time of penetration, and to determine the incubation period of *S. sorghi.*

MATERIALS AND METHODS.—Two methods were used to infect grain sorghum with *S. sorghi:* (i) artificial inoculation; and (ii) exposure to conidia in the field. Test plants used were cultivars Pioneer 846 and DeKalb C48A grown in greenhouse flats to the 2- to 3-leaf stage. Inoculation was accomplished by placing infected leaves on top of seedlings and incubating at high relative humidity. Infected leaves used as a source of inoculum were removed from young, systemically infected plants immediately before use. The infected leaves were placed on top of the healthy seedlings in contact with the foliage. Flats containing the seedlings were enclosed overnight in polyethylene bags. A flat of each cultivar without inoculum was maintained for a check. Conidia formed on inoculum leaves during the night and were discharged onto the seedling leaves by early morning. Leaves were removed from inoculated seedlings at hourly intervals from 7:00 to 10:00 AM, and submerged in beakers of lactophenol. Also at 8:00 AM, leaves were removed from which freehand sections were cut.

For exposure to conidia in the field, two flats of seedlings, one of each cultivar, were placed in the field late in the afternoon beside infected grain sorghum plants. The next morning, leaves were removed from these seedlings at hourly intervals from 7:00 to 10:00 AM and treated as mentioned above.

For microscopic examination of penetration and infection, leaves which had been placed in lactophenol were first cleared by autoclaving in the lactophenol for 10 min. Whole leaf mounts were then prepared in lactophenol or lactophenol-cotton blue (1). Freehand sections were cleared by heating in lactophenol and mounted in lactophenol-cotton blue. Observations were made under phase contrast and bright field at ×200-1,000 magnification.

RESULTS.—Conidia formed during the night were released and germinated, and penetration occurred in the leaves of both cultivars through open stomata. Germ tubes grew at random on the leaf surface. When they crossed junctions between epidermal cells, irregular-shaped swellings developed at or near these junctions (Fig. 1-A). Penetration was not observed at the site of these swellings. Germ tube elongation continued until a stoma was encountered or until energy for growth was exhausted. When an open stoma was reached, an appressorium formed over the stomatal opening (Fig. 1-B). No penetration peg was apparent. Instead, a small vesicle formed immediately inside the stomatal opening. This vesicle enlarged to ultimately form a large oval-shaped vesicle within the substomatal cavity (Fig. 1C, D, E). Numerous substomatal vesicles were attached to germ tubes without appressoria, indicating that appressoria are not always formed (Fig. 1-E). The substomatal vesicle gave rise to one or more infection hyphae (Fig. 1-F). Haustoria formed by infection hyphae entered nearby mesophyll cells (Fig. 1-G).

Examination of inoculated leaves removed at hourly intervals and fixed in lactophenol showed that penetration and the establishment of infection is rapid. Penetration was completed on leaves removed at 7:00 AM from seedlings in either the greenhouse or the field. Infection hyphae were formed by 9:00 AM, and haustorial formation was completed by 10:00 AM.

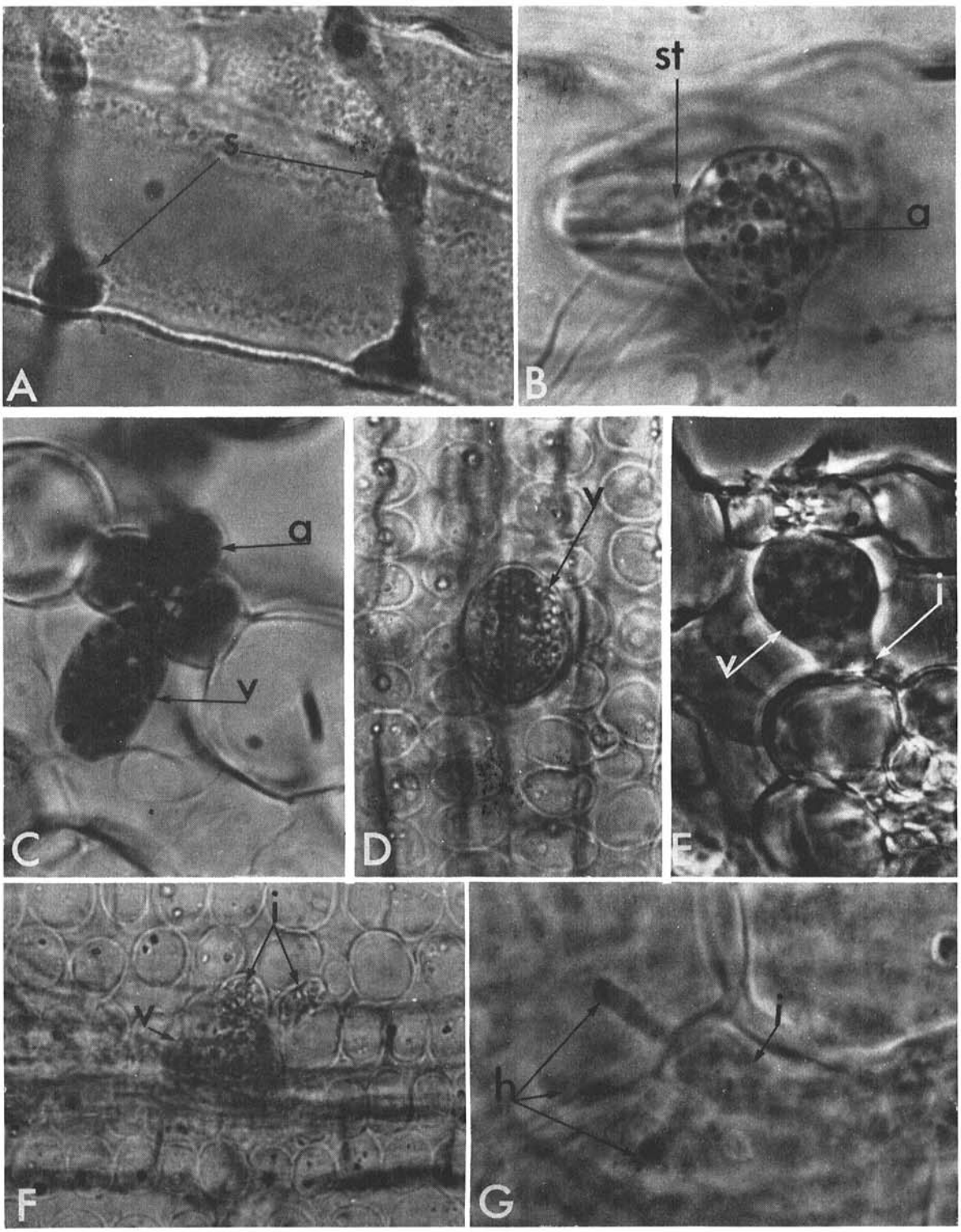

Fig. 1. *Sclerospora sorghi* penetration and infection in *Sorghum bicolor* leaves. **A)** Germ tubes on the surface of a leaf showing swellings at epidermal cell junctions (×500). **B)** An appressorium (a) over a stoma (st) (×700). **C)** Vesicle (v) protruding into substomatal cavity. Note appressorium (a) over stomatal opening (×900). **D)** Fully developed vesicle (v) as it appears when viewed beneath the stoma in a whole leaf mount (×400). **E)** Infection hyphal initial (i) extending from substomatal vesicle (v); note germ tube without an appressorium (×600). **F)** Infection hyphae (i) extending from substomatal vesicle (v). The hyphae developed by 9:00 AM following early morning penetration (×200). **G)** Infection hypha (i) with three haustoria (h) within mesophyll cells. Development of haustoria occurred by 10:00 AM following early morning penetration (×1,000).

Disease symptoms developed in seedlings of both cultivars 6 days after inoculation. Sporulation occurred 2 days later. No symptoms developed on the checks.

DISCUSSION.—Previous investigators found that *S. sorghi* forms conidia about midnight (5) or during the early hours of the morning (10). Kenneth (6) reported that *S. sorghi* conidia captured from 2 to 4 AM were capable of germinating within 1 hr, and could germinate for as long as 6 hr unless the temp rose. He also reported that conidia held in a moist chamber throughout the day failed to germinate the night after their formation. In the present study, some of the infected leaves used to provide inoculum had conidia which were formed previous to use; others did not. Conidia formed during the night on the latter, as well as the former, germinated, and penetration occurred by 7 AM. The conidia were discharged onto seedlings and the soil below the seedlings in the absence of air movement, indicating that they were discharged by force. It is the opinion of the writer that conidia of *S. sorghi* which function as inoculum are forcefully discharged soon after their formation. Those remaining attached to conidiophores lose their viability rapidly during the day and become nonfunctional as inoculum. Consequently, in order to spread to new host plants by means of conidia, the pathogen must form and discharge conidia, reach a new host, and penetrate prior to the drying of the foliage. This could account for the difficulty encountered by researchers (10, 11, 12) in obtaining artificial infection when conidia were used as inoculum. It is feasible that conidia brushed from conidiophores had already lost their viability, or did so soon after their removal.

Swelling of germ tubes at epidermal cell junctions may represent attempts at penetration. Although direct penetration was not observed, it is possible that under different environmental conditions it may occur. This assumption is based on the fact that direct and stomatal penetration has been observed with other Peronosporales (3, 9).

The penetration mechanism of *S. sorghi* is similar to that reported for *Peronospora destructor* in onion leaves (13). Yarwood also noted that *P. destructor* occasionally penetrated without first forming an appressorium.

If one considers the presence of infection hyphae with haustoria as evidence of infection (8), then the time period for *S. sorghi* conidial formation, germination, penetration, and infection is about 10 hr.

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